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(54) Title: NOVEL FATTY ACID ANALOGOUS

(57) Abstract: The present invention relates to novel fatty acid analogues of the general formula (I): $R_1 - [x_i - CH_2]_n - COOR_2$, wherein R_1 is: a C_6 - C_{24} alkene with one or more double bonds and/or with one or more triple bonds, and/or a C_6 - C_{24} alkyne, and/or a C_6 - C_{24} alkyl substituted in one or several positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C_1 - C_4 alkoxy, C_1 - C_4 alkylthio, C_2 - C_3 acyloxy or C_1 - C_4 alkyl, and wherein R_2 represents hydrogen or C_1 - C_4 alkyl, and wherein n is an integer from 1 to 12, and wherein i is an odd number and indicates the position relative to $COOR_2$, and wherein X_i independent of each other are selected from the group comprising O, S, SO, SO₂, Se and CH₂, and with the proviso that at least one of the X_i is not CH₂, and with the proviso that if R_1 is an alkyne, then the carbon-carbon triple bond is positioned between the (ω -1) carbon and the (ω -2) carbon, or between the (ω -2) carbon and the (ω -3) carbon, or between the (ω -3) carbon and the (ω -4) carbon, a salt, prodrug or complex thereof, which can be used for the treatment and/or prevention of syndrome X, obesity, hypertension, fatty liver, diabetes, hyperglycaemia, hyperinsulinaemia and stenosis. Further, the invention relates to a nutritional composition comprising said fatty acid analogues, and a method for reducing the total weight, or the amount of adipose tissue in an animal.

NOVEL FATTY ACID ANALOGOUS.

FIELD OF THE INVENTION

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The present invention relates to novel fatty acid analogous. Further, the invention relates to the use of the novel fatty acid analogous for the treatment and/or prevention of syndrome X, obesity, hypertension, fatty 10 liver, diabetes, hyperglycaemia, hyperinsulinemia and stenosis. The invention also relates to processes for the preparation of the novel fatty acid analogues.

15 BACKGROUND OF THE INVENTION

EP 345.038 describes the use of non- β -oxidizable fatty acid analogues of the formula;

20

 $\text{Alkyl-X-CH}_2\text{COOR}$

wherein the alkyl is a saturated or unsaturated hydrocarbon chain of 8 to 22 carbon atoms, X represents a O, S, SO or SO₂, and R is hydrogen or a C1 - C4 alkyl group, for the 25 treatment of hyperlipaemic conditions and for the reducing the concentration of cholesterol and triglycerides in the blood of mammals.

PCT/NO95/00195 describes alkyl-S-CH₂COOR and alkyl-Se-CH₂COOR for the inhibition of the oxidative modification of LDL. Further, this application describes the use of the selenium-compound for the treatment of hyperlipaemic condition and for reducing the concentration of cholesterol and triglycerides.

The PCT applications PCT/NO99/00135, PCT/NO99/00136 and PCT/NO99/00149 describes fatty acid analogous of the formula (I)



- wherein n is an integer from 1 to 12, and
- wherein m is an integer from 0 to 23, and
- wherein i is an odd number which indicates the
10 position relative to COOR, and
- wherein X_i independent of each other are selected
 from the group comprising O, S, SO, SO₂, Se and CH₂,
 and
- wherein R represents hydrogen or C₁-C₄ alkyl,
15 - with the proviso that at least one of the X_i is not
 CH₂,
 or a salt, prodrug or complex thereof.

This formula comprises one or several X groups (preferably
20 selenium and sulphur) in positions 3, 5, 7, 9, etc.

Further, these PCT applications describe several medicinal
and nutritional applications.

25 PCT/NO99/00135 describes the use of the fatty acid
 analogues the treatment and/or prevention of obesity,
 hypertension, fatty liver and the multi metabolic syndrome
 termed «metabolic syndrome» or Syndrome X. Further, this
 application describes a method for the treatment or
30 prevention of an obese or overweight condition, and a
 method for producing weigh loss or a reduction of the fat
 mass in a human or non-human animal. The application also
 describes a nutritional composition effective to reduce, or

to prevent an increase in, the total body weight or the total body fat mass in a human or non-human animal, and also a method for the modification of the fat distribution and content of animals in order to improve the quality of 5 the meat, or product such as milk and eggs.

PCT/NO99/00136 describes use of fatty acid analogues for the treatment and/or prevention of diabetes (both type I and II), and a method for the treatment or prevention of 10 hyperglycaemia, hyperinsulinemia and reduced sensitivity to insulin. A nutritional composition effective to reduce, or to prevent an increase in the concentration of glucose in the blood of a human or non-human animal is also disclosed, as is a method for reducing the concentration of glucose in 15 the blood of a human or non-human animal.

PCT/NO99/00149 describes the use of the fatty acid analogues for the treatment and/or prevention of primary and/or secondary stenosis, and/or a disease caused by 20 procedural vascular trauma and/or pathological proliferation of smooth muscle cells, and/or an increased level of plasma homocysteine.

Due to the X-atom (most preferable sulphur or selenium) 25 that is substituted in the carbon chain of the above given fatty acid analogues, these compounds will not be β -oxidized in the mitochondria beyond this position. Thus, the degradation of these molecules must start from the methyl end of the fatty acid, and this is a rather slow 30 metabolic process. The catabolism of these fatty acid analogues includes ω -oxidation and chain shortening of the dicarboxylic acid by peroxisomes. Enzymes in the endoplasmic reticulum will ω -hydroxylate and further oxidise the hydroxylated fatty acid to a dicarboxylic acid.

This acid may then be chain shortened by β -oxidation in the peroxisomes. Studies in rats have shown that 50% of the analogue TTA was excreted in the urine as short sulfoxy dicarboxylic acids within 24 hours of administration. In 5 similar experiments it has been found that a desaturated product of TTA is formed in vivo. This is due to the microsomal enzyme Δ^9 -desaturase which inserts a double bond in the 9-position of saturated fatty acids.

10 It is anticipated that this desaturated product has similar effects, and/or mediates the biological effects of the saturated fatty acid analogues. It is also likely that the biological effects of fatty acid analogues may be potentiated by slowing down their catabolism. This can be 15 done by inserting double and/or triple bonds near the methyl end of the fatty acids, and/or by incorporating alkyl groups or halogens in this part of the molecule. Such molecules, i.e. the compounds in accordance with the present invention, will not be substrates for the relevant 20 microsomal enzymes.

OBESITY, AND RELATED DISEASES

Obesity is a chronic disease that is highly prevalent in 25 modern society and is associated not only with a social stigma, but also with decreased life span and numerous medical problems, including adverse psychological development, reproductive disorders such as polycystic ovarian disease, dermatological disorders such as infections, 30 varicose veins, Acanthosis nigricans, and eczema, exercise intolerance, diabetes mellitus, insulin resistance, hypertension, hypercholesterolemia, cholelithiasis, osteoarthritis, orthopedic injury, thromboembolic disease, cancer, and coronary heart disease.

It is therefore an object of the present invention to provide a treatment regimen that is useful in returning the body weight of obese subjects toward a normal, ideal body weight.

It is another object to provide a therapy for obesity that results in maintenance of the lowered body weight for an extended period of time. Further, it is an object to reduce or inhibit the weight gain normally induced by fat rich diets.

It is yet another object to prevent obesity and, once treatment has begun, to arrest progression or prevent the onset of diseases that are the consequence of, or secondary to, the obesity, such as hypertension and fatty liver. These and other objects will be apparent to those of ordinary skill in the art.

The obesity herein may be due to any cause, whether genetic or environmental. Examples of disorders that may result in obesity or be the cause of obesity include overeating and bulimia, polycystic ovarian disease, craniopharyngioma, the Prader-Willi Syndrome, Frohlich's syndrome, Type II diabetics, GH-deficient subjects, normal variant short stature, Turner's syndrome, and other pathological conditions showing reduced metabolic activity.

It is also an object of the present invention to provide a treatment regimen that is useful in lowering the blood pressure.

Further, it is an object of the present invention to provide a treatment regimen that is useful in lowering the

concentration of triacylglycerols in the liver. It is anticipated that such a regimen will provide an inhibiting effect on the development of a fatty liver condition, and also be suited as a method for the treatment of the 5 manifested disease.

The compounds of the present invention activate the β -oxidation, and also reduce the concentration of triglycerides in the liver.

10

The term "metabolic syndrome" is used to describe a multi-metabolic syndrome which is *inter alia* characterized by hyperinsulinemia, insulin resistance, obesity, glucose intolerance, Type 2 diabetes mellitus, dyslipidemia or 15 hypertension.

As indicated above it is anticipated that the compounds of the present invention will provide a positive effect on all the conditions mentioned above, i.e. by regulating both the 20 glucose and lipid homeostasis, and thus it is anticipated that the compounds of the present invention will be suitable agents for the regulation of the above defined metabolic disease (sometimes called syndrome X).

25 DIABETES

There are two major forms of diabetes mellitus. One is type I diabetes, which is also known as insulin-dependent diabetes mellitus (IDDM), and the other is type II 30 diabetes, which is also known as noninsulin-dependent diabetes mellitus (NIDDM). Most patients with IDDM have a common pathological picture; the nearly total disappearance of insulin-producing pancreatic beta cells which results in hyperglycemia.

Considerable evidence has been accumulated showing that most IDDM is the consequence of progressive beta-cell destruction during an asymptomatic period often extending , 5 over many years. The prediabetic period can be recognized by the detection of circulating islet-cell autoantibodies and insulin autoantibodies.

There is a need for a compound which would be nontoxic and 10 have no side effects but which would prevent clinical IDDM and NIDDM.

Type I diabetes: severe diabetes mellitus, usually of abrupt onset prior to maturity, characterized by low plasma 15 insulin levels, polydipsia, polyuria, increased appetite, weight loss and episodic ketoacidosis; also referred to as IDDM.

Type II diabetes: an often mild form of diabetes mellitus, 20 often of gradual onset, usually in adults, characterized by normal to high absolute plasma insulin levels which are relatively low in relation to plasma glucose levels; also referred to as NIDDM.

25 Type I and II diabetes are in accordance with an etiologic classification considered as «primary» diabetes respectively.

Secondary diabetes comprises pancreatic, extrapancreatic/ 30 endocrine or drug-induced diabetes. Further, some types of diabetes are classified as exceptional forms. These include lipoatrophic, myotonic diabetes, and a type of diabetes caused by disturbance of insulin receptors.

Considering the high prevalence of diabetes in our society and the serious consequences associated therewith as discussed above, any therapeutic drug potentially useful for the treatment and prevention of this disease could have 5 a profound beneficial effect on their health. There is a need in the art for a drug that will reduce the concentration of glucose in the blood of diabetic subjects without significant adverse side effects.

10 It is therefore an object of the present invention to provide a treatment regimen that is useful in lowering the blood glucose and to treat a diabetic condition.

15 It is yet another object of the invention to provide a treatment regimen that is useful in lowering the concentration of insulin in the blood, and to increase the effect of the remaining insulin.

STENOSIS

20 Many pathological conditions have been found to be associated with smooth muscle cell proliferation. Such conditions include restenosis, arteriosclerosis, coronary heart disease, thrombosis, myocardial infarction, stroke, 25 smooth muscle neoplasms such as leiomyoma and leiomyosarcoma of the bowel and uterus and uterine fibroid or fibroma.

30 Over half a million interventional intravascular procedures are performed each year. While such invasive procedures continue to improve over time, as many as 30-50% of the procedures performed each year fail as a result of restenosis, i.e. the formation of secondary stenosis. The reduction of restenosis is, therefore, often cited as the

most critical factor in increasing the success realised in the treatment of cardiovascular disease through the use of interventional intravascular procedures, such as angioplasty, atherectomy, and procedures utilising stents and 5 laser technology.

In balloon angioplasty, e.g. Percutaneous Transluminal Coronary Angioplasty (PTCA), a small incision is made to an artery in the patient's leg or arm and a long hollow tube, 10 called a guide catheter, is inserted into the artery. A thick guide wire and deflated balloon catheter are then inserted into the guide catheter and are carefully advanced through the patient's blood vessels using x-ray visualization. The deflated balloon is advanced until it 15 reaches the site of the luminal narrowing, at which point the physician inflates the balloon one or more times to a pressure of about 4-6 atm for about 60 sec. When inflated, the balloon cracks and fractures the plaque and stretches the muscle fibre in the artery wall beyond its ability to 20 recoil completely. Although no plaque is removed in this procedure, the fracturing of the plaque and the stretching of the arterial wall increase the vessel lumen, thereby allowing for increased blood flow.

25 The restenosis that accompanies such procedures is characterised by platelet aggregation and adhesion, smooth muscle cell proliferation, narrowing of the vessel lumen, restricted vasodilatation, and an increase in blood pressure. Smooth muscle cells in the intimal layer of the 30 artery have been reported to enter the growth cycle within about 2-3 days of these procedures and to proliferate for several days thereafter (intimal hyperplasia).

Compounds that reportedly suppress smooth muscle prolife-

ration in vitro may have undesirable pharmacological side effects when used in vivo. Heparin is an example of one such compound, which reportedly inhibits smooth muscle cell proliferation in vitro but when used in vivo has the 5 potential adverse side effect of inhibiting coagulation.

As is apparent from the foregoing, many problems remain to be solved in the use of inhibitory drugs to effectively treat smooth muscle cell mobilisation and proliferation. It 10 would be highly advantageous to develop new compositions or methods for inhibiting stenosis, restenosis or related disorders due to proliferation and mobilisation of vascular smooth muscle cells following, for example, traumatic injury to vessels rendered during vascular surgery.

15

It is anticipated that the compounds in accordance with the present invention will be effectively in the treatment of these diseases.

20

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel fatty acid analogues of the general formula (I):

25



- wherein R_1 is;

- a C₆-C₂₄ alkene with one or more double bonds and/or with one or more triple bonds, and/or

30 - a C₆-C₂₄ alkyne, and/or

- a C₆-C₂₄ alkyl substituted in one or several positions with one or more compounds selected

from the group comprising fluoride, chloride, hydroxy, C₁-C₄ alkoxy, C₁-C₄ alkylthio, C₂-C₅ acyloxy or C₁-C₄ alkyl, and

5 - wherein R₂ represents hydrogen or C₁-C₄ alkyl, and

- wherein n is an integer from 1 to 12, and

10 - wherein i is an odd number and indicates the position relative to COOR₂, and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO₂, Se and CH₂, and

15

- with the proviso that at least one of the X_i is not CH₂, and

20

- with the proviso that if R₁ is an alkyne, then the carbon-carbon triple bond is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon, or between the (ω-3) carbon and the (ω-4) carbon,

or a salt, prodrug or complex thereof.

25

Another aspect of the present invention relates to novel fatty acid analogues wherein the R moiety comprises one carbon-carbon double bond. A preferred embodiment relates to a fatty acid analogue wherein the carbon-carbon double bond is in the 9-position and in a cis configuration.

30 Most preferred embodiments of the present invention relates

to compounds of formula (I) wherein a sulphur or selenium is arranged in position 3.

5 Further, the invention also relates to processes for the preparation of a compound of the formula (I), wherein the compound is prepared as described in example 1.

The present invention also relates to the use of the compound of formula (I) as pharmaceutical and/or 10 nutritional agents. It is anticipated that the present compounds will exhibit substantially the same biological activities as the prior art compounds described above, and the present invention thus relates to the use of the present compounds of formula (I) for the applications 15 described in the indicated publications.

Thus, the present invention relates to the use of the compounds of the formula (I) for the preparation of a pharmaceutical composition for the treatment and/or 20 prevention of a condition selected from the group comprising syndrome X, obesity, hypertension, fatty liver, diabetes, hyperglycaemia, hyperinsulinemia and stenosis.

Further, the invention relates to the use of a compound of 25 the formula (I) for:

- lowering the concentration of cholesterol and triglycerides in the blood of mammals,
- inhibiting the oxidative modification of low density lipoprotein

30 The present invention also relates to a nutritional composition of formula (I), effective to reduce, or to prevent an increase in the total body weight or the total body fat mass in a human or non-human animal, and a method

for producing weigh loss or a reduction of the fat mass in a human or non-human animal in need thereof.

FIGURE LEGENDS

5

Figure 1 shows a scheme for the synthesis of the compound (Z) 3-Thia-heptadec-9-enoic-acid.

10 Figure 2 shows a scheme for the synthesis of 3-Thia-15-heptadecyne.

ADMINISTRATION OF THE COMPOUNDS OF THE PRESENT INVENTION

15 As a pharmaceutical medicament the compounds of the present invention may be administered directly to the animal by any suitable technique, including parenterally, intranasally, orally, or by absorption through the skin. They can be administered locally or systemically. The specific route of 20 administration of each agent will depend, e.g., on the medical history of the animal.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention.

25

EXPERIMENTAL SECTION

METHODS

5 The methods described below were used as test systems for the compounds described in the prior art, and are thus also be used to test the biological effects of the present compounds.

10 Obese Zucker (fa/fa) rats.

The obese Zucker (fa/fa) rats used in this study were bred at the U 465 INSERM animal facility from pairs originally provided by the Harriet G. Bird Laboratory (Stow, MA, USA). Unless otherwise stated, the animals were maintained under 15 a constant light-dark cycle (light from 7:00 a.m. to 7:00 p.m.) at 21±1 °C and were given free access to food and water. Three rats were housed per cage. Weight gains were recorded daily.

20 Wistar rats

Male Wistar Charles River rats weighing 280-358 were purchased from AnLab Ltd. (Prague, Czech Republic) and housed in wire-mesh cages in a temperature (22±1 °C) and light-controlled (light from 7.00 a.m. to 7.00 p.m.) room. 25 They were given free access to chow and water. Three rats were housed per cage. Weight gain and food intake were recorded daily.

Intravenous glucose tolerance tests

30 Male Zucker (fa/fa) rats (5 weeks old) were anaesthetised after a 5-hours fast, by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The rats were injected with glucose (0.55 g/kg) in the saphenous vein and blood samples were collected from the tail vein in heparinized

tubes at time 0, 5, 10, 15, 20 and 30 minutes after the glucose load. Samples were kept on ice, centrifuged and plasma was stored at -20 °C until analysis.

5 Hyperinsulinemic euglycemic clamp.

After 21 days on their respective diets (see above), the rats were anaesthetised by injection of xylazine hydrochloride (Rometar SPOFA, Prague, Czech Republic; 10 mg/ml) and ketamine hydrochloride (Narkamon SPOFA, Prague, Czech Republic; 75 mg/ml), and fitted with chronic carotid artery and jugular vein cannulas as described by Koopmans et al. (Koopmans, S.J., et al., *Biochim Biophys Acta*, 1115, 2130-2138 1992.). The cannulated rats were allowed to recover for two days after surgery before the clamping studies 15 which were carried out according to Kraegen et al. (Kraegen, E. W., et al., *Am J Physiol*, 248, E353-E362 1983.). Thus, on the third day after surgery, unrestrained conscious rats were given a continuous infusion of porcine insulin (Actrapid, Novo Nordisk, Denmark) at a dose of 6.4 20 mU per kg per min to achieve plasma insulin levels in the upper physiological range. The arterial blood glucose concentration was clamped at the basal fasting level, by variable infusion of a 30 % w/v glucose solution (Leciva, Prague, Czech Republic). Blood samples for determination of 25 plasma glucose and insulin concentrations were obtained every 15 minutes from the start of the glucose infusion. After 90 minutes, the rats were disconnected from the infusions and immediately decapitated, blood was collected for plasma separation, liver and epididymal adipose tissue 30 pads were dissected out and weighed.

Measurement of plasma parameters

Glucose (GLU, Boehringer Mannheim, Germany), free fatty acids (NEFA, C ACS-ACOD kit; Wako Chemicals, Dalton, USA)

and β -hydroxybutyrate (310-A kit; Sigma Diagnostics Inc., St. Louis, USA) concentrations were measured using enzymatic methods. Insulin concentrations were determined with radioimmunoassay by (CIS bio International, Gif sur Yvette, France) using rat insulin as standard in the Zucker rats. In the Wistar Charles River rats, plasma glucose concentrations were measured with the aid of Beckman Glucose Analyzer (Fullerton, CA, USA). Plasma insulin levels were measured using a RIA kit from Linco Research Inc. (St. Charles, MO, USA). Phospholipids were measured by the enzymatic method of bioMérieux, Marcy-l'Etoile, France, Triacylglycerol by the Technicon Method no. SA4-0324L90, USA and Cholesterol by the Technicon Method no. SA4-0305L90, USA.

15

Preparation of post-nuclear and mitochondrial fractions and measurement of enzyme activities

Freshly isolated livers from individual old Zucker rats, were homogenised in ice-cold sucrose buffer (0.25 M sucrose, 10 mM HEPES (pH 7.4) and 2 mM EDTA). Post-nuclear and mitochondrial fractions were prepared using preparative differential centrifugation according to DeDuve et al. (De Duve, C., et al., Biochem. J., 60, 604-617 1955.) Modifications, purity and yield were as described earlier (Garras, A., et al., Biochim. Biophys. Acta, 1255, 154-160 1995.). Acid soluble products were measured in post-nuclear and mitochondrial enriched fractions, using [$1-^{14}\text{C}$]-palmitoyl-CoA and [$1-^{14}\text{C}$]-palmitoyl-L-carnitine (Radiochemical Centre, Amersham, England) as substrates as described earlier (Willumsen, N., et al., J. Lipid Res., 34, 13-22 1993. Carnitine palmitoyltransferase-I and -II activities were measured in the post-nuclear and mitochondrial fractions essentially as described by Bremer (Bremer, J., Biochim. Biophys. Acta, 665, 628-631 1981.)

and 3-hydroxy-3-methylglutharyl-CoA synthase was measured according to Clinkenbeard et al. (Clinkenbeard, K. D., et al., J. Biol. Chem., 250, 3108-3116 1975.) in the mitochondrial fractions.

5

RNA analysis

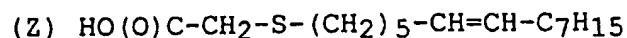
RNA extraction (Chomczynski, P., et al., Anal. Biochem., 162, 156-159 1987.), Northern blot analysis and slot blotting of RNA onto nylon filters, and hybridisation to immobilised RNA were performed as earlier described (Vaagenes, H., et al., Biochem. Pharmacol., 56, 1571-1582 1998.). The following cDNA fragments were used as probes: CPT-I, (Esser, V. et al., J. Biol. Chem., 268, 5817-5822 1993), CPT-II (Woeltje, K. F., et al., J. Biol. Chem., 265, 10720-10725 1990.), 3-hydroxy-3-methylglutharyl-CoA synthase (Ayté, J., et al., Proc. Natl. Acad. Sci. USA., 87, 3874-3878 1990.), and hormone sensitive lipase (Holm, C., et al., Biochim. Biophys. Acta, 1006, 193-197 1989.). The relative levels of RNA expression were estimated as the amounts of radioactive probe hybridised to the respective levels of 28S rRNA.

RESULTS

25 Example 1. Synthesis of novel fatty acid compounds

A) Non- β -oxidizable fatty acid analogous with a carbon-carbon double bond.

30 The synthesis of a compound in accordance with the present invention is representatively elaborated with reference to the synthesis of the thia-heptadec-9-enoic acid;



(Z) designates a *cis* configuration.

1. Preparation of 1-bromo-5-hydroxy-pentane

5

Pentane-1,5-diol, HO-(CH₂)₅-OH, was treated with HBr in benzene and refluxed for 24 h. The product mixture was chromatographed first with a 85:15 hexane-diethyl ether mixture to remove the dibromide and then with a 70:30 mixture. Yield of 1-bromo-5-hydroxy-pentane, 80 %.

10

¹H-NMR: 1,81(-CH₂-CH₂OH), 1,44(-CH₂-), 3,35(-CH₂-Br),
3,55(-CH₂-OH), 3,32(-OH), 1,51(-CH₂-CH₂Br).

15

¹³C-NMR: 31,43-32,30(C₂,C₄), 24,24(C₃), 33,64(C₅),
62,11(C₁).

20 2. Preparation of 5-(tetrahydropyranloxy)-1-bromopentane

This compound was allowed to react with 3,4-dihydro-2H-pyran in CH₂Cl₂ at 0 °C. 2 drops of conc. HCl was used as catalyst. After removal of the solvent the reaction product was chromatographed in 95:5 hexane-diethyl ether. The yield of 5-(tetrahydropyranloxy)-1-bromopentane was 77 %.

25
30 ¹H-NMR: 1,45-1,63(-CH₂-), 1,83(-CH₂-CH₂O-), 3,38(-CH₂-Br),
3,27-3,79(-CH₂-O-), 4,52(-O-CH-O).

¹³C-NMR: 24,9-32,92(C₂-C₄), 33,61(C₅), 62,26(C₆), 98,83 (C₁

in THP).

3. Preparation of 7-(tetrahydropyranloxy)-1-heptyne

5 The product from step 2 was treated with the EDA complex of Li-acetylide in dry dimethyl sulfoxide at 0 °C under argon. After 4 h at room temperature the reaction mixture was hydrolysed with water and organic products extracted with diethyl ether. The residue after removing the ether was 10 chromatographed in 97:3 hexane-diethyl ether, yielding 7-(tetrahydropyranloxy)-1-heptyne in 62 % yield.

15 $^1\text{H-NMR}$: 1,45-1,66(-CH₂-), 3,45-3,82(-CH₂-O), 2,16(-CH₂-C≡), 1,90(HC≡C-), 4,53(-O-CH-O-).

$^{13}\text{C-NMR}$: 18,27-30,66(C₃-C₆), 62,21(C₇), 68,14(C₁), 84,40(C₂).

20

4. Preparation of 1-(tetrahydropyranloxy)-tetradec-6-yne

To a 1,6 M solution of BuLi in hexane dissolved in THF at 0 °C under argon and the product from 3 was added a mixture 25 of 1-bromoheptane and N,N-dimethylpropyleneurea. After hydrolysis, extraction and chromatography 1-(tetrahydro-pyranloxy)-tetradec-6-yne was isolated in 69 % yield.

30 $^1\text{H-NMR}$: 0,85(CH₃-), 1,22-1,57(-CH₂-), 2,10(-CH₂-C≡), 3,30-3,84(-CH₂O-), 4,55(-O-CH-O-).

^{13}C -NMR: 14,02 (C₁₄), 22,60-31,73 (C₂-C₁₃), 18,69-18,71 (C₅ og C₈), 62,23 (C₁), 79,91-80,32 (C₆ og C₇).

5 5. Preparation of 1(Tetrahydropyranloxy)-tetradec-6-ene

The substituted tetradec-6-yne from step 4 was reduced with hydrogen in the presence of the Lindlar catalyst in ethanol. The reduction lasted for 4 h. 1(Tetrahydro-10 pyranloxy)-tetradec-6-ene appeared sufficiently pure for step 6 without further purification but could be isolated in 89 % yield after chromatography.

^1H -NMR: 0,90 (CH₃-), 1,27-1,61 (-CH₂-), 3,39-3,89 (-CH₂-O), 15 2,04 (-CH₂-C=), 4,59 (-O-CH-O-), 5,37 (-HC=CH-).

^{13}C -NMR: 14,07 (C₁₄), 22,65-31,85 (C₂-C₁₃), 62,27 (C₁), 27,13, 27,19 (C₅ and C₈), 129,60-130,04 (C₆ and C₇).

20

6. Preparation of 1-bromotetradec-6-ene

The product from 5 was brominated with CBr₄ at 0 °C in dichloromethane in the presence of Ph₃P. The reaction 25 mixture was stirred overnight. The yield of 1-bromotetradec-6-ene was quantitative.

^1H -NMR: 0,87 (CH₃-), 1,27-1,52 (-CH₂-), 2,01 (-CH₂-C=), 30 3,39 (-CH₂-Br), 1,45 (-CH₂-CH₂-Br), 1,85 (-CH₂-CH₂C=), 5,34 (-HC=CH-).

¹³C-NMR: 14,00 (C₁₄), 22,60-32,68 (C₂-C₁₃), 26,97, 27,24 (C₅ and C₈), 33,75 (C₁), 129,15-130,32 (C₆ and C₇).

5

7. Preparation of (Z) thia-heptadec-9-enoic acid.

The bromodecene from step 6 in methanol was added to 3 equivalents of KOH and 1.5 equivalents of HS-CH₂-C(O)OH in 10 methanol under argon during 30 min. After stirring at room temperature for 4 h, refluxing for another 12 h, followed by hydrolysis and extraction with diethyl ether, then acidifying to pH 1-2, the product, the title compound, was isolated as viscous oil in 60 % yield.

15

The following analyses have been performed; IR, 600 MHz ¹H and ¹³C NMR, MS, GC, GC-MS of the methyl ester. The NMR results are given below. All data are given in parts per million (ppm). No trace of the E-compound could be 20 detected.

¹H-NMR : 0.86 (CH₃-), 1.16-1.60 (-CH₂-), 1.99 (-CH₂-C=), 2.64 (-CH₂-S-), 3.22 (-S-CH₂-C(O)OH), 5.33 (HC=CH).

25 ¹³C-NMR : 176.63 (C₁), 33.34 (C₂), 32.69 (C₄), 22.63-31.83 (C₅-C₇, C₁₂-C₁₆), 129.32 and 130.24 (C₉, C₁₀), 26.98 and 27.19 (C₈, C₁₁), 14.08 (C₁₇).

B) Non- β -oxidizable fatty acid analogous comprising a 30 carbon-carbon triple binding.

The synthesis of a compound in accordance with the present invention is representatively elaborated with reference to

the synthesis of the 3-Thia-15-heptadecyne, as given in figure 1.

5 1. Preparation of 11-Bromo-1(tetrahydro-2-pyranyloxy)undecane.

Pyridine toluene 4-sulphonate (1,0 g, 4,0 mmol) and 11-Bromo-1-undecanol (10,0 g, 400 mmol) were dissolved in dry 10 CH₂CH₂ (200 ml) at ambient temperature, and 3,4-dihydro-2H-pyran (5,0 g, 60 mmol) was added. The reaction mixture was stirred overnight. The crude product was purified by flash chromatography on silica gel eluted with CH₂Cl₂. The yield of 11-Bromo-1(tetrahydro-2-pyranyloxy)undecane was 10,7 g 15 (80%).

20 2. Preparation of 14-(tetrahydro-2-pyranoyl)-2-tetradecyne.

Propyne gas was bubbled through a solution of MeLi in diethyl ether (0,8 M, 60 ml, 51,2 mmol) in a rate adapted to ensure reflux of the ether. When there were no longer any heat development, the reaction was considered finished 25 (white slurry). 11-Bromo-1(tetrahydro-2-pyranyloxy)undecane (product 2) (13,0 g, 38,8 mmol) was added drop by drop to this solution over a period of 20 minutes. The reaction was stirred overnight, and water (50 ml) was carefully added drop by drop. The mixture was diluted with 30 diethyl ether and washed with water (5x), dried (MgSO₄) and the solvent was evaporated off. The crude product was purified by flash chromatography with CH₂Cl₂ as eluent. The yield of 14-(tetrahydro-2-pyranoyl)-2-tetradecyne was 8,5 g (74%).

3. Preparation of 12-Tetradecyn-1-ol

5 Pyridine Toluene 4-Sulphonate (0,3 g, 1,2 mmol) and the alkyne (product 3) were dissolved in ethanol (25 ml) and heated to 50 °C overnight. The solvent was evaporated and distributed between water and CH₂Cl₂. The water phase was washed with water, dried (MgSO₄) and the solvent was 10 evaporated. The crude product was purified with flash chromatography with CH₂Cl₂ as eluent. The yield of 12-Tetradecyn-1-ol was 1,5 g (78%).

15 4. Preparation of 14-Bromo-2-tetradecyne

12-Tetradecyn-1-ol (5,0, 23,8 mmol) was dissolved in hexane (50ml) and 10 drops of pyridine was added. PBr₃ was added to this mixture. The mixture was heated to 60 °C for three 20 hours, cooled, and water was added drop by drop. The mixture was washed by water, dried (MgSO₄) and the solvent was evaporated. The crude product was purified with flash chromatography with hexane as eluent until 2,5 % EtOAc in hexane. The yield of 14-Bromo-2-tetradecyne was 2,2 g 25 (34%).

5. Preparation of 3-Thia-15-heptadecyne

30 KOH (2,76 g, 49,0 mmol) was dissolved in methanol (30 ml), and thioglycolic acid (2,04 g, 22,1 mmol) in methanol (25 ml) was added drop by drop. After 10 minutes the 14-Bromo-2-tetradecyne (5,5 g, 20,1 mmol) was carefully added drop by drop, and the mixture was heated to 50 °C overnight. The

mixture was cooled to 0°C, and 30 ml HCl was added (pH = 1). The precipitate was filtered and washed with water (2x). The solid material was dissolved in chloroform (100 ml) and washed with water (1x), dried ($MgSO_4$) and the solvent was evaporated off. The yield of the compound 14-Bromo-2-tetradecyne was 4,4 g (77 %).

¹⁰ ¹H NMR (300 MHz, $CDCl_3$) δ : 1.26 (10 H, sharp m), 1.3-1.4 (4 H, m), 1.46 (2 H, quint, J = 7.0 Hz, $\equiv CCH_2CH_2-$), 1.60 (2 H, quint, J = 7.0 Hz, $-CH_2CH_2S-$), 1.77 (3 H, t, J = 2.6 Hz, $CH_3C\equiv$), 2.10 (2 H, tq, J = 2.6, 7.0 Hz, $\equiv CCH_2-$), 2.65 (2 H, t, J = 7.3 Hz, $-CH_2S-$), 3.25 (2 H, s, $-SCH_2COOH$), 10.40 (1 H, broad s, $-COOH$).

¹⁵ ¹³C NMR (75 MHz, $CDCl_3$) δ : 3.35 ($CH_3C\equiv$), 18.61 ($\equiv CCH_2-$), 28.50, 28.78, 28.78, 28.97, 29.04, 29.04, 29.34, 29.38, 29.40, 32.70 ($-CH_2CH_2S-$), 33.3'' ($-SCH_2CO$), 75.20 ($MeC\equiv C-$), 79.31 ($MeC\equiv C-$), 176.42 (CO).

²⁰ C) Non- β -oxidizable fatty acid analogous substituted in one or several positions.

²⁵ One or several of the hydrogen groups of the fatty acid chain can be substituted with one or more of the compounds selected from the group comprising fluoride, chloride, hydroxy, C_1-C_4 alkoxy, C_1-C_4 alkylthio, C_2-C_5 acyloxy or C_1-C_4 alkyl. The substituents can for instance be incorporated in the formula (I) compound by selecting other substrates in the steps 1-4 above.

³⁰ Finally, the compounds prepared in step (C) above can be converted to saturated compounds with a traditionally hydrogenation reaction, thus giving an R1 group which is fully saturated (i.e. an alkyl), but substituted at one or ³⁵ more positions.

Example 2Toxicity study of TTA

5

Toxicity studies, and test for mutagenic activity will be performed as described in PCT/NO99/00135.

Example 3.

10

The biological activity of the novel compounds in accordance with the present invention will be determined as described in the experimental section above, or as disclosed in the publications cited above.

15

CLAIMS

1. Novel fatty acid analogues of the general formula (I):

5



- wherein R_1 is;

- a C₆-C₂₄ alkene with one or more double bonds
and/or with one or more triple bonds, and/or

10

- a C₆-C₂₄ alkyne, and/or

- a C₆-C₂₄ alkyl substituted in one or several
positions with one or more compounds selected
from the group comprising fluoride, chloride,
hydroxy, C₁-C₄ alkoxy, C₁-C₄ alkylthio, C₂-C₅

15

acyloxy or C₁-C₄ alkyl, and

- wherein R₂ represents hydrogen or C₁-C₄ alkyl, and

- wherein n is an integer from 1 to 12, and

20

- wherein i is an odd number and indicates the
position relative to COOR₂, and

25

- wherein X_i independent of each other are selected
from the group comprising O, S, SO, SO₂, Se and CH₂,
and

- with the proviso that at least one of the X_i is not
CH₂, and

- with the proviso that if R1 is an alkyne, then the carbon-carbon triple bond is positioned between the (ω -1) carbon and the (ω -2) carbon, or between the (ω -2) carbon and the (ω -3) carbon, or between the (ω -3) carbon and the (ω -4) carbon,

5

or a salt, prodrug or complex thereof.

2. Novel fatty acid analogues in accordance with claim 1,
10 wherein the R moiety comprises one carbon-carbon triple bond.

3. Novel fatty acid analogues in accordance with claim 1,
15 wherein the R moiety comprises one carbon-carbon double bond.

4. Novel fatty acid analogues in accordance with claim 5,
wherein said carbon-carbon double bond is in the 9-
position.

20 5. Novel fatty acid analogues in accordance with claim 6,
wherein the carbon-carbon double bond is in a *cis* configuration.

25 6 Novel fatty acid analogues in accordance with one of
the claims 1-5, wherein the $X_i=3$ is sulphur.

7. Novel fatty acid analogues in accordance with one of
the claims 1-5, wherein $X_i=3$ is selenium.

30

8. Process for the preparation of a compound of the formula (I), wherein the compound is prepared as described in example 1.

9. Use of a compound of the formula (I)



5 - wherein R₁ is;

- a C₆-C₂₄ alkene with one or more double bonds and/or with one or more triple bonds, and/or
- a C₆-C₂₄ alkyne, and/or
- a C₆-C₂₄ alkyl substituted in one or several 10 positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C₁-C₄ alkoxy, C₁-C₄ alkylthio, C₂-C₅ acyloxy or C₁-C₄ alkyl, and

15 - wherein R₂ represents hydrogen or C₁-C₄ alkyl, and

- wherein n is an integer from 1 to 12, and
- wherein i is an odd number and indicates the 20 position relative to COOR₂, and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO₂, Se and CH₂, and

25 - with the proviso that at least one of the X_i is not CH₂, and

- with the proviso that if R₁ is an alkyne, then the

carbon-carbon triple bond is positioned between the (ω -1) carbon and the (ω -2) carbon, or between the (ω -2) carbon and the (ω -3) carbon, or between the (ω -3) carbon and the (ω -4) carbon,

5

or a salt, prodrug or complex thereof, for the preparation of a pharmaceutical composition for the treatment and/or prevention of a condition selected from the group comprising syndrome X, obesity, hypertension, fatty liver, 10 diabetes, hyperglycaemia, hyperinsulinemia and stenosis.

10. Use of a compound of the formula (I)



15

- wherein R_1 is;

- a C₆-C₂₄ alkene with one or more double bonds and/or with one or more triple bonds, and/or

- a C₆-C₂₄ alkyne, and/or

20

- a C₆-C₂₄ alkyl substituted in one or several positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C₁-C₄ alkoxy, C₁-C₄ alkylthio, C₂-C₅ acyloxy or C₁-C₄ alkyl, and

25

- wherein R₂ represents hydrogen or C₁-C₄ alkyl, and

- wherein n is an integer from 1 to 12, and

30

- wherein i is an odd number and indicates the

position relative to COOR_2 , and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO_2 , Se and CH_2 , and

- with the proviso that at least one of the X_i is not CH_2 , and

- with the proviso that if R_1 is an alkyne, then the carbon-carbon triple bond is positioned between the ($\omega-1$) carbon and the ($\omega-2$) carbon, or between the ($\omega-2$) carbon and the ($\omega-3$) carbon, or between the ($\omega-3$) carbon and the ($\omega-4$) carbon,

15 or a salt, prodrug or complex thereof, for the preparation of a pharmaceutical composition for;

- lowering the concentration of cholesterol and triglycerides in the blood of mammals,

20 - inhibiting the oxidative modification of low density lipoprotein

11. A nutritional composition comprising fatty acid analogues of the general formula (I);

25



- wherein R_1 is;

- a C₆-C₂₄ alkene with one or more double bonds and/or with one or more triple bonds, and/or

30 - a C₆-C₂₄ alkyne, and/or

- a C₆-C₂₄ alkyl substituted in one or several

positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C₁-C₄ alkoxy, C₁-C₄ alkylthio, C₂-C₅ acyloxy or C₁-C₄ alkyl, and

5

- wherein R₂ represents hydrogen or C₁-C₄ alkyl, and

- wherein n is an integer from 1 to 12, and

10

- wherein i is an odd number and indicates the position relative to COOR₂, and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO₂, Se and CH₂,

15

and

- with the proviso that at least one of the X_i is not CH₂, and

20

- with the proviso that if R₁ is an alkyne, then the carbon-carbon triple bond is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon, or between the (ω-3) carbon and the (ω-4) carbon,

25

or a salt, prodrug or complex thereof, effective to reduce, or to prevent an increase in, the total body weight or the total body fat mass in a human or non-human animal.

30

12. A method for producing weigh loss or a reduction of the fat mass in a human or non-human animal in need thereof, comprising administering thereto an effective amount of a composition comprising fatty acid analogues of

the general formula (I):



- wherein R_1 is;

5 - a C₆-C₂₄ alkene with one or more double bonds
and/or with one or more triple bonds, and/or

- a C₆-C₂₄ alkyne, and/or

- a C₆-C₂₄ alkyl substituted in one or several
10 positions with one or more compounds selected
from the group comprising fluoride, chloride,

hydroxy, C₁-C₄ alkoxy, C₁-C₄ alkylthio, C₂-C₅
acyloxy or C₁-C₄ alkyl, and

- wherein R_2 represents hydrogen or C₁-C₄ alkyl, and

15 - wherein n is an integer from 1 to 12, and

- wherein i is an odd number and indicates the
position relative to COOR₂, and

20 - wherein X_i independent of each other are selected
from the group comprising O, S, SO, SO₂, Se and CH₂,
and

25 - with the proviso that at least one of the X_i is not
CH₂, and

- with the proviso that if R_1 is an alkyne, then the
carbon-carbon triple bond is positioned between the
(ω -1) carbon and the (ω -2) carbon, or between the (ω -

2) carbon and the (ω -3) carbon, or between the (ω -3) carbon and the (ω -4) carbon,
or a salt, prodrug or complex thereof.

1/2

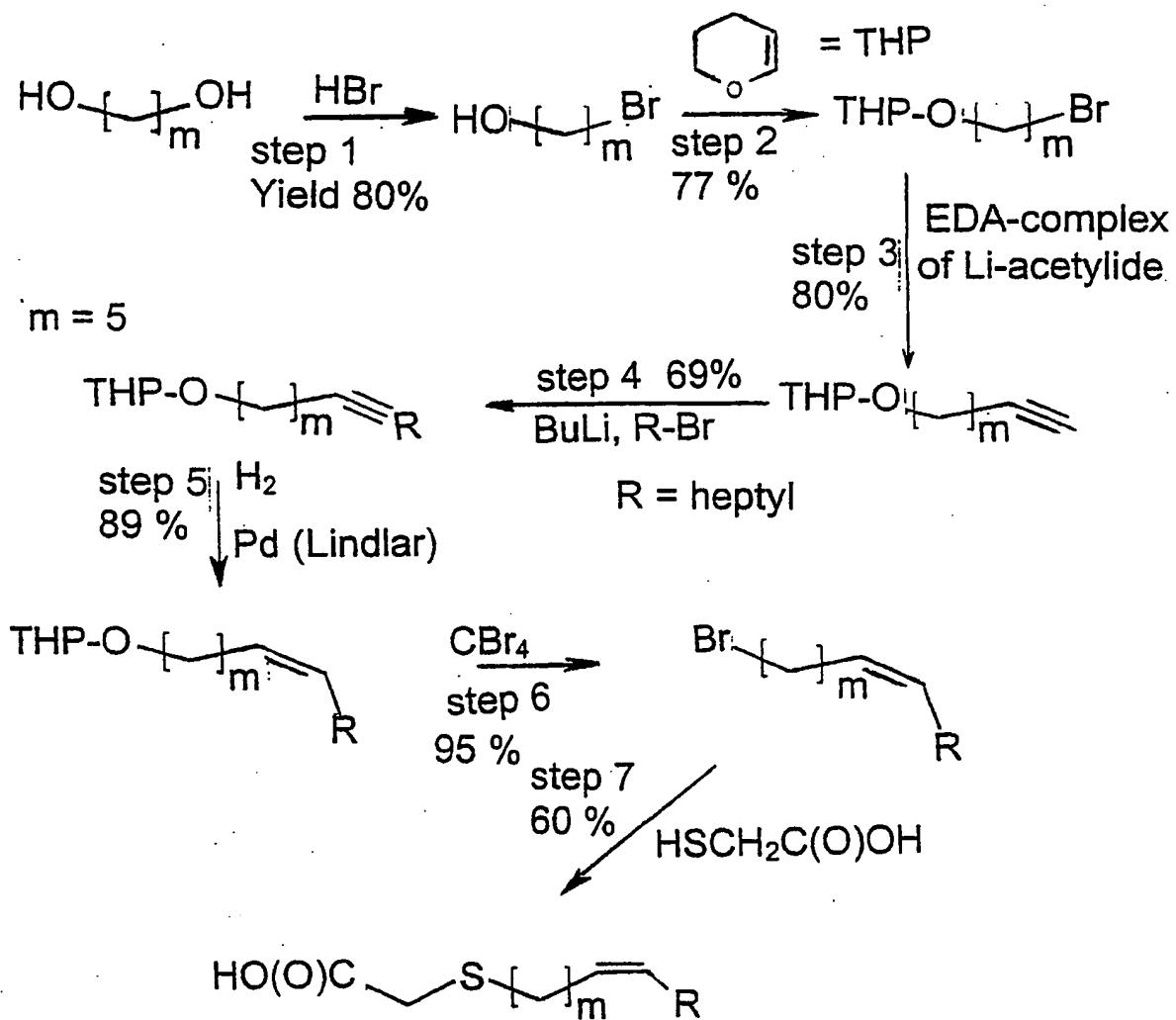


Figure 1

2/2

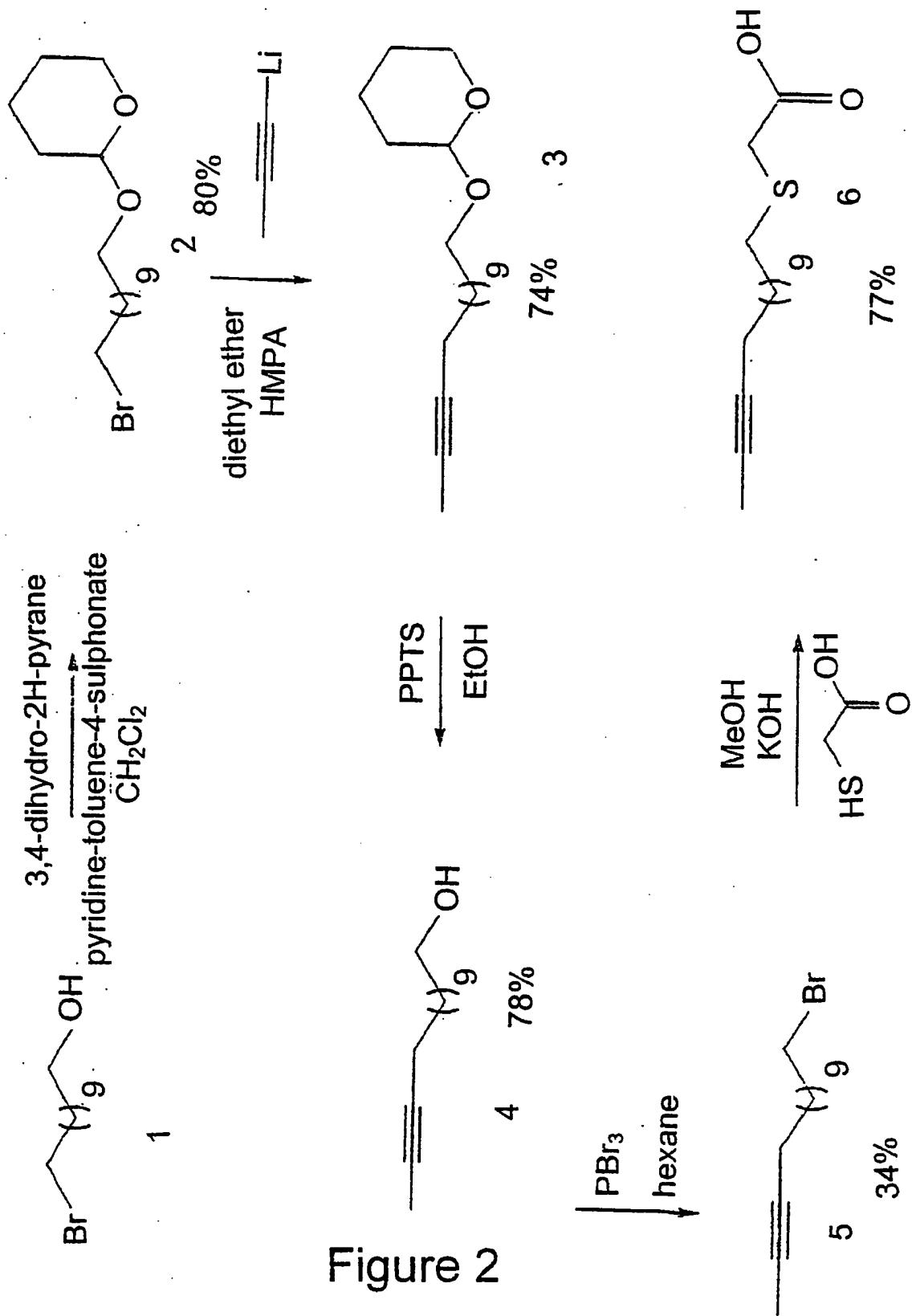


Figure 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 01/00082

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07C 57/03, C07C 57/18, C07C 321/18, A61K 31/20, A61K 31/201, A61P 3/00, A61P 5/50, A61P 9/10, A61P 9/12
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07C, A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, EPO-INTERNAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0345038 A2 (NORSK HYDRO A.S.), 6 December 1989 (06.12.89), page 3 - page 4, abstract, examples 1-5 --	1-7,9-12
X	WO 9703663 A1 (BERGE, ROLF ET AL), 6 February 1997 (06.02.97), page 6 - page 8, abstract, examples 1-2 --	1-7,9-12
X	WO 9958120 A1 (BERGE, ROLF), 18 November 1999 (18.11.99), page 6, line 5 - line 15, abstract, experiments, p. 9-17 --	1-7,9-12

 Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent not published on or after the international filing date
- "I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search
18 June 2001

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 01/00082

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9958121 A1 (THIA MEDICA AS), 18 November 1999 (18.11.99), page 22, abstract, table --	1-7,9-12
X	WO 9958122 A1 (THIA MEDICA AS), 18 November 1999 (18.11.99), page 19, abstract, table --	1-7,9-12
X	WO 9958123 A2 (THIA MEDICA AS), 18 November 1999 (18.11.99), page 15, abstract, table --	1-7,9-12
X	WO 9611908 A1 (PEPTIDE TECHNOLOGY LIMITED ET AL), 25 April 1996 (25.04.96), page 35, claim 18, abstract, examples, p 16-26 --	1-7
X	EP 0342115 A1 (CENTRE INTERNATIONAL DE RECHERCHES DERMATOLOGIQUES C.I.R.D.), 15 November 1989 (15.11.89), page 10 - page 17, abstract, examples --	1-7
X	EP 0622370 A2 (ROUSSEL-UCLAF), 2 November 1994 (02.11.94), page 12, line 45 - page 13, line 7 --	1-7
X	US 3910790 A (KARL LOHMER ET AL), 7 October 1975 (07.10.75), table 4, compound 31 --	1-7
X	J. Org. Chem., Volume 36, No 15, 1971, Kikumasa Sato et al, "A Synthesis of Dihydrothiopyran-3-ones. The Intramolecular Cyclization of Allylthioglycolic Acid Chlorides", page 2077 - page 2080, compound 3a-d --	1-7

INTERNATIONAL SEARCH REPORT

International application No. PCT/NO 01/00082
--

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. CHEM. RESEARCH(S), 1990, Makoto Yamamoto et al, "Cyclisation of Alkynecarboxylic Acids: Synthesis and Reactions of 6-Methylene-1,4-oxathian-2-ones and Their 4,4-Dioxides", page 12-13, compound 3a-3e --	1-7
X	J. CHEM. RESEARCH (M), 1990, 0273-0281, compound 3a-e --	1-7
X	J. Chem. Soc. Perkin Trans.1, 1999, Teresa M.V.D. Pinho e Melo et al, "Intramolecular dipolar cyclo-addition reaction of 5H,7H-thiazolo(3,4-c)oxazol-4-1-olates: synthesis of chiral 1H-pyrrolo(1,2-c)thiazole derivatives", page 1219 - page 1223, see page 1221 --	1-7
P,X	EP 1044966 A1 (L'OREAL), 18 October 2000 (18.10.00) --	1-7
A	Biochemical Pharmacology, Volume 51, 1996, Pengfei Wu et al, "Effects of Chain Length and Sulphur Position of Thia Fatty Acids on Their Incorporation into Phospholipids in 7800 C1 Hepatoma Cells and Isolated Rat Hepatocytes, and Their Effects on Fatty Acid Composition of", page 751 - page 758 -- -----	1-12

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/NO01/00082**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: **12**
because they relate to subject matter not required to be searched by this Authority, namely:
See extra sheet
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO01/00082

Claim 12 relate to a method of treatment of the human or animal body by surgery or by therapy/a diagnostic methods practised on the human or animal body/ Rule. 39.1.(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the compound(s)/composition(s).

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/05/01

International application No.

PCT/NO 01/00082

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0345038 A2	06/12/89	SE 0345038 T3 AT 96664 T 15/11/93 CA 1329550 A 17/05/94 DE 68910386 D, T 09/06/94 DK 267689 A 03/12/89 ES 2059749 T 16/11/94 GB 8813012 D 00/00/00 US 5093365 A 03/03/92	
WO 9703663 A1	06/02/97	AU 4272696 A 18/02/97 CA 2226871 A 06/02/97 EP 0840604 A 13/05/98 JP 11514339 T 07/12/99 NO 952796 D 00/00/00 US 6046237 A 04/04/00	
WO 9958120 A1	18/11/99	AU 4936699 A 29/11/99 AU 4936799 A 29/11/99 AU 5451799 A 29/11/99 AU 7240398 A 29/11/99 EP 1075258 A 14/02/01 EP 1075259 A 14/02/01 EP 1075260 A 14/02/01 NO 20005461 A 08/01/01 NO 20005462 A 08/01/01 NO 20005463 A 08/01/01 WO 9958121 A 18/11/99 WO 9958122 A 18/11/99 WO 9958123 A 18/11/99	
WO 9958121 A1	18/11/99	AU 4936699 A 29/11/99 AU 4936799 A 29/11/99 AU 5451799 A 29/11/99 AU 7240398 A 29/11/99 EP 1075258 A 14/02/01 EP 1075259 A 14/02/01 EP 1075260 A 14/02/01 NO 20005461 A 08/01/01 NO 20005462 A 08/01/01 NO 20005463 A 08/01/01 WO 9958120 A 18/11/99 WO 9958122 A 18/11/99 WO 9958123 A 18/11/99	

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/05/01

International application No.

PCT/NO 01/00082

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 9958122 A1	18/11/99		AU 4936699 A AU 4936799 A AU 5451799 A AU 7240398 A EP 1075258 A EP 1075259 A EP 1075260 A NO 20005461 A NO 20005462 A NO 20005463 A WO 9958120 A WO 9958121 A WO 9958123 A	29/11/99 29/11/99 29/11/99 29/11/99 14/02/01 14/02/01 14/02/01 08/01/01 08/01/01 08/01/01 18/11/99 18/11/99 18/11/99
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